

SEQUENCE LISTING

<110> TOYO BOSEKI KABUSHIKI KAISHA

<120> MODIFIED THERMOSTABLE DNA POLYMERASE

<130> 000053

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<150> 2000-138796

<151> 2000-05-11

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<170> PatentIn Ver. 2.1

<210> 1

<211> 5342

<212> DNA

<213> Pyrococcus kodakaraensis

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<222> (156)..(5165)

<223> 1374-2453 intron, 2709-4316 intron

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cctggattgt tctacaagat tatgggggat gaaag atg atc ctc gac act gac 173

Met Ile Leu Asp Thr Asp

1

5

tac ata acc gag gat gga aag cct gtc ata aga att ttc aag aag gaa 221

Tyr Ile Thr Glu Asp Gly Lys Pro Val Ile Arg Ile Phe Lys Lys Glu

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15

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aac ggc gag ttt aag att gag tac gac cgg act ttt gaa ccc tac ttc 269

Asn Gly Glu Phe Lys Ile Glu Tyr Asp Arg Thr Phe Glu Pro Tyr Phe

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tac gcc ctc ctg aag gac gat tct gcc att gag gaa gtc aag aag ata 317

Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile Glu Glu Val Lys Lys Ile

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acc gcc gag agg cac ggg acg gtt gta acg gtt aag cgg gtt gaa aag 365

Thr Ala Glu Arg His Gly Thr Val Val Thr Val Lys Arg Val Glu Lys

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gtt cag aag aag ttc ctc ggg aga cca gtt gag gtc tgg aaa ctc tac 413

Val Gln Lys Lys Phe Leu Gly Arg Pro Val Glu Val Trp Lys Leu Tyr

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ttt act cat ccg cag gac gtc cca gcg ata agg gac aag ata cga gag 461

Phe Thr His Pro Gln Asp Val Pro Ala Ile Arg Asp Lys Ile Arg Glu

T.007F.50, 22625860

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cat ggg gca gtt att gac atc tac gag tac gac ata ccc ttc gcc aag 509			
His Gly Ala Val Ile Asp Ile Tyr Glu Tyr Asp Ile Pro Phe Ala Lys			
105	110	115	
cgc tac ctc ata gac aag gga tta gtg cca atg gaa ggc gac gag gag 557			
Arg Tyr Leu Ile Asp Lys Gly Leu Val Pro Met Glu Gly Asp Glu Glu			
120	125	130	
ctg aaa atg ctc gcc ttc gac att gaa act ctc tac cat gag ggc gag 605			
Leu Lys Met Leu Ala Phe Asp Ile Glu Thr Leu Tyr His Glu Gly Glu			
135	140	145	150
gag ttc gcc gag ggg cca atc ctt atg ata agc tac gcc gac gag gaa 653			
Glu Phe Ala Glu Gly Pro Ile Leu Met Ile Ser Tyr Ala Asp Glu Glu			
155	160	165	
ggg gcc agg gtg ata act tgg aag aac gtg gat ctc ccc tac gtt gac 701			
Gly Ala Arg Val Ile Thr Trp Lys Asn Val Asp Leu Pro Tyr Val Asp			
170	175	180	
gtc gtc tcg acg gag agg gag atg ata aag cgc ttc ctc cgt gtt gtg 749			
Val Val Ser Thr Glu Arg Glu Met Ile Lys Arg Phe Leu Arg Val Val			
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295 300 305 310

315 320 325

330 335 340

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360 365 370

375 380 385 390

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Glu Pro Glu Arg Gly Leu Trp Glu Asn Ile Val Tyr Leu Asp Phe Arg

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tgc cat cca gcc gat acg aag gtt gtc gtc aag ggg aag ggg att ata 1421

Cys His Pro Ala Asp Thr Lys Val Val Val Lys Gly Lys Gly Ile Ile

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aac atc agc gag gtt cag gaa ggt gac tat gtc ctt ggg att gac ggc 1469

Asn Ile Ser Glu Val Gln Glu Gly Asp Tyr Val Leu Gly Ile Asp Gly

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tgg cag aga gtt aga aaa gta tgg gaa tac gac tac aaa ggg gag ctt 1517

Trp Gln Arg Val Arg Lys Val Trp Glu Tyr Asp Tyr Lys Gly Glu Leu

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gta aac ata aac ggg tta aag tgt acg ccc aat cat aag ctt ccc gtt 1565

Val Asn Ile Asn Gly Leu Lys Cys Thr Pro Asn His Lys Leu Pro Val

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gtt aca aag aac gaa cga caa acg aga ata aga gac agt ctt gct aag 1613

Val Thr Lys Asn Glu Arg Gln Thr Arg Ile Arg Asp Ser Leu Ala Lys

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Ser Phe Leu Thr Lys Lys Val Lys Gly Lys Ile Ile Thr Thr Pro Leu

00552922 051001

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att tca cac cag tat cgt gtt gag ata acc att ggg aaa gac gag gag 1853

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gag ttt agg gat cgt atc aca tac att ttt gag cgt ttg ttt ggg att 1901

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act cca agc atc tgc gag aag aaa gga act aac gca gta aca ctc aaa 1949

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ata ctg ttc caa aca ctc att gga ttc atc agt gaa aga aag aac gct 2285

Ile Leu Phe Gln Thr Leu Ile Gly Phe Ile Ser Glu Arg Lys Asn Ala

695 700 705 710

ctg ctt aat aag gca ata tct cag agg gaa atg aac aac ttg gaa aac 2333

Leu Leu Asn Lys Ala Ile Ser Gln Arg Glu Met Asn Asn Leu Glu Asn

715 720 725

aat gga ttt tac agg ctc agt gaa ttc aat gtc agc acg gaa tac tat 2381

Asn Gly Phe Tyr Arg Leu Ser Glu Phe Asn Val Ser Thr Glu Tyr Tyr

730 735 740

gag ggc aag gtc tat gac tta act ctt gaa gga act ccc tac tac ttt 2429

Glu Gly Lys Val Tyr Asp Leu Thr Leu Glu Gly Thr Pro Tyr Tyr Phe

745 750 755

gcc aat ggc ata ttg acc cat aac tcc ctg tac ccc tca atc atc atc 2477

Ala Asn Gly Ile Leu Thr His Asn Ser Leu Tyr Pro Ser Ile Ile Ile

760 765 770

acc cac aac gtc tcg ccg gat acg ctc aac aga gaa gga tgc aag gaa 2525

Thr His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu

775 780 785 790

tat gac gtt gcc cca cag gtc ggc cac cgc ttc tgc aag gac ttc cca 2573

09852922 051001

795 800 805

Gly Phe Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys

810 815 820

Ile Lys Lys Lys Met Lys Ala Thr Ile Asp Pro Ile Glu Arg Lys Leu

825 830 835

Leu Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Ile Leu

840 845 850

Pro Glu Glu Trp Leu Pro Val Leu Glu Glu Gly Glu Val His Phe Val

855 860 865 870

Arg Ile Gly Glu Leu Ile Asp Arg Met Met Glu Glu Asn Ala Gly Lys

875 880 885

Val Lys Arg Glu Gly Glu Thr Glu Val Leu Glu Val Ser Gly Leu Glu

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gtc ccg tcc ttt aac agg aga act aac aag gcc gag ctc aag aga gta			2909
Val Pro Ser Phe Asn Arg Arg Thr Asn Lys Ala Glu Leu Lys Arg Val			
905	910	915	
aag gcc ctg att agg cac gat tat tct ggc aag gtc tac acc atc aga			2957
Lys Ala Leu Ile Arg His Asp Tyr Ser Gly Lys Val Tyr Thr Ile Arg			
920	925	930	
ctg aag tcg ggg agg aga ata aag ata acc tct ggc cac agc ctc ttc			3005
Leu Lys Ser Gly Arg Arg Ile Lys Ile Thr Ser Gly His Ser Leu Phe			
935	940	945	950
tct gtg aga aac ggg gag ctc gtt gaa gtt acg ggc gat gaa cta aag			3053
Ser Val Arg Asn Gly Glu Leu Val Glu Val Thr Gly Asp Glu Leu Lys			
955	960	965	
cca ggt gac ctc gtt gca gtc ccg cgg aga ttg gag ctt cct gag aga			3101
Pro Gly Asp Leu Val Ala Val Pro Arg Arg Leu Glu Leu Pro Glu Arg			
970	975	980	
aac cac gtg ctg aac ctc gtt gaa ctg ctc ctt gga acg cca gaa gaa			3149
Asn His Val Leu Asn Leu Val Glu Leu Leu Leu Gly Thr Pro Glu Glu			
985	990	995	

gaa act ttg gac atc gtc atg acg atc cca gtc aag ggt aag aag aac 3197

Glu Thr Leu Asp Ile Val Met Thr Ile Pro Val Lys Gly Lys Lys Asn

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ttc ttt aaa ggg atg ctc agg act ttg cgc tgg att ttc gga gag gaa 3245

Phe Phe Lys Gly Met Leu Arg Thr Leu Arg Trp Ile Phe Gly Glu Glu

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aag agg ccc aga acc gcg aga cgc tat ctc agg cac ctt gag gat ctg 3293

Lys Arg Pro Arg Thr Ala Arg Arg Tyr Leu Arg His Leu Glu Asp Leu

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ggc tat gtc cgg ctt aag aag atc ggc tac gaa gtc ctc gac tgg gac 3341

Gly Tyr Val Arg Leu Lys Lys Ile Gly Tyr Glu Val Leu Asp Trp Asp

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tca ctt aag aac tac aga agg ctc tac gag gcg ctt gtc gag aac gtc 3389

Ser Leu Lys Asn Tyr Arg Arg Leu Tyr Glu Ala Leu Val Glu Asn Val

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aga tac aac ggc aac aag agg gag tac ctc gtt gaa ttc aat tcc atc 3437

Arg Tyr Asn Gly Asn Lys Arg Glu Tyr Leu Val Glu Phe Asn Ser Ile

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Val Lys Leu Asp Lys Lys Lys Asn Ala Tyr Tyr Ser His Val Ile Pro

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aag gaa gtc ctg agc gag gtc ttt ggg aag gtt ttc cag aaa aac gtc			4109
Lys Glu Val Leu Ser Glu Val Phe Gly Lys Val Phe Gln Lys Asn Val			
1305	1310	1315	
agt cct cag acc ttc agg aag atg gtc gag gac gga aga ctc gat ccc			4157
Ser Pro Gln Thr Phe Arg Lys Met Val Glu Asp Gly Arg Leu Asp Pro			
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gaa aag gcc cag agg ctc tcc tgg ctc att gag ggg gac gta gtg ctc			4205
Glu Lys Ala Gln Arg Leu Ser Trp Leu Ile Glu Gly Asp Val Val Leu			
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gac cgc gtt gag tcc gtt gat gtg gaa gac tac gat ggt tat gtc tat			4253
Asp Arg Val Glu Ser Val Asp Val Glu Asp Tyr Asp Gly Tyr Val Tyr			
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gac ctg agc gtc gag gac aac gag aac ttc ctc gtt ggc ttt ggg ttg			4301
Asp Leu Ser Val Glu Asp Asn Glu Asn Phe Leu Val Gly Phe Gly Leu			
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Val Tyr Ala His Asn Ser Tyr Tyr Gly Tyr Tyr Gly Tyr Ala Arg Ala			
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1495 1500 1505 1510

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Pro Gly Thr Val Ile Ser Tyr Ile Val Leu Lys Gly Ser Gly Arg Ile

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Gly Asp Arg Ala Ile Pro Phe Asp Glu Phe Asp Pro Thr Lys His Lys

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Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln Val Leu Pro Ala Val Glu

1625

1630

1635

aga att ctg aga gcc ttc ggt tac cgc aag gaa gac ctg cgc tac cag 5117

Arg Ile Leu Arg Ala Phe Gly Tyr Arg Lys Glu Asp Leu Arg Tyr Gln

1640

1645

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aag acg aga cag gtt ggt ttg agt gct tgg ctg aag ccg aag gga act 5165

Lys Thr Arg Gln Val Gly Leu Ser Ala Trp Leu Lys Pro Lys Gly Thr

1655

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1665

1670

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〈213〉 *Pyrococcus kodakaraensis*

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Thr Phe Glu Pro Tyr Phe Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile

35 40 45

Glu Glu Val Lys Lys Ile Thr Ala Glu Arg His Gly Thr Val Val Thr

50 55 60

Val Lys Arg Val Glu Lys Val Gln Lys Lys Phe Leu Gly Arg Pro Val

65 70 75 80

Glu Val Trp Lys Leu Tyr Phe Thr His Pro Gln Asp Val Pro Ala Ile

85 90 95

Arg Asp Lys Ile Arg Glu His Pro Ala Val Ile Asp Ile Tyr Glu Tyr

100

105

110

Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Val Pro

115

120

125

Met Glu Gly Asp Glu Glu Leu Lys Met Leu Ala Phe Asp Ile Glu Thr

130

135

140

Leu Tyr His Glu Gly Glu Glu Phe Ala Glu Gly Pro Ile Leu Met Ile

145

150

155

160

Ser Tyr Ala Asp Glu Glu Gly Ala Arg Val Ile Thr Trp Lys Asn Val

165

170

175

Asp Leu Pro Tyr Val Asp Val Val Ser Thr Glu Arg Glu Met Ile Lys

180

185

190

Arg Phe Leu Arg Val Val Lys Glu Lys Asp Pro Asp Val Leu Ile Thr

195

200

205

Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Cys Glu

210

215

220

Lys Leu Gly Ile Asn Phe Ala Leu Gly Arg Asp Gly Ser Glu Pro Lys

09552993 051004
FOOTER 222550

225 230 235 240

Ile Gln Arg Met Gly Asp Arg Phe Ala Val Glu Val Lys Gly Arg Ile

245 250 255

His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr

260 265 270

Tyr Thr Leu Glu Ala Val Tyr Glu Ala Val Phe Gly Gln Pro Lys Glu

275 280 285

Lys Val Tyr Ala Glu Glu Ile Thr Thr Ala Trp Glu Thr Gly Glu Asn

290 295 300

Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr

305 310 315 320

Glu Leu Gly Lys Glu Phe Leu Pro Met Glu Ala Gln Leu Ser Arg Leu

325 330 335

Ile Gly Gln Ser Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu

340 345 350

Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala

355 360 365

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Pro Asn Lys Pro Asp Glu Lys Glu Leu Ala Arg Arg Arg Gln Ser Tyr

370

375

380

Glu Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Glu Asn Ile

385

390

395

400

Val Tyr Leu Asp Phe Arg Ser Leu Tyr Pro Ser Ile Ile Ile Thr His

405

410

415

Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp

420

425

430

Val Ala Pro Gln Val Gly His Arg Phe Cys Lys Asp Phe Pro Gly Phe

435

440

445

Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys

450

455

460

Lys Lys Met Lys Ala Thr Ile Asp Pro Ile Glu Arg Lys Leu Leu Asp

465

470

475

480

Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly Tyr

485

490

495

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Tyr Gly Tyr Ala Arg Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser

500

505

510

Val Thr Ala Trp Gly Arg Glu Tyr Ile Thr Met Thr Ile Lys Glu Ile

515

520

525

Glu Glu Lys Tyr Gly Phe Lys Val Ile Tyr Ser Asp Thr Asp Gly Phe

530

535

540

Phe Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala

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550

555

560

Met Glu Phe Leu Lys Tyr Ile Asn Ala Lys Leu Pro Gly Ala Leu Glu

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Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys Lys

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Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu

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Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala

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620

Arg Val Leu Glu Ala Leu Leu Lys Asp Gly Asp Val Glu Lys Ala Val

09852922.054001

625 630 635 640

Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro

645 650 655

Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Lys Asp

660 665 670

Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala

675 680 685

Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu

690 695 700

Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Phe Asp Glu Phe

705 710 715 720

Asp Pro Thr Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln

725 730 735

Val Leu Pro Ala Val Glu Arg Ile Leu Arg Ala Phe Gly Tyr Arg Lys

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Glu Asp Leu Arg Tyr Gln Lys Thr Arg Gln Val Gly Leu Ser Ala Trp

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Leu Lys Pro Lys Gly Thr

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<213> Artificial Sequence

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<223> Description of Artificial Sequence:primer

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<223> Description of Artificial Sequence:primer

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<223> Description of Artificial Sequence:primer

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<223> Description of Artificial Sequence:primer

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<223> Description of Artificial Sequence:primer

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<210> 10

TOP SECRET

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<213> Artificial Sequence

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<223> Description of Artificial Sequence:primer

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<213> Artificial Sequence

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